CHAPTER 1

General introduction
Outline of the thesis

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Urinary albumin excretion

Interest in the value of low levels of urinary albumin excretion (UAE) dates back to 1981, when it was shown that concentrations of urinary albumin that could not be detected by the standard dipsticks at that moment predict development of overt proteinuria in patients with diabetes mellitus.[1;2] It was described that in such patients a urinary albumin excretion (UAE) between 30 and 140 mg/min had a 24 fold higher risk to develop nephropathy. A few years later, in 1984, it was also shown that such low levels of albuminuria predict mortality in patients with diabetes.[3] Since, the term "microalbuminuria" has been introduced, reflecting the interest that rose in the clinical value of such slightly elevated levels of UAE.

In adults the normal mean value for UAE is 10 mg per day.[4] However, UAE can be slightly increased under certain physiological circumstances, such as upright posture, exercise, pregnancy, and fever. Therefore, it is generally recommended in case an abnormal test result is obtained to confirm this on two separate occasions. The present definitions of microalbuminuria were described by the seventh report of the Joint National Committee (JNC 7) in 2002 and summarized in Table 1.

	Urine collection method	Normal	microalbuminuria	macroalbuminuria
Albumin	24-hour excretion	<30 mg/day	30-300 mg/day	>300 mg/day
	Spot urine albumin-Specific Dipstick	<20 mg/L	>20-200 mg/L	>200 mg/L
	Spot urine albumin-to creatinine ratio	<17 mg/g (♂) <25 mg/g(♀)	17-250 mg/g (♂) 25-355 mg/g (♀)	>250 mg/g (♂) >355 mg/g (♀)

Table 1. Definitions of albuminuria according to JNC 7 [4]

The glomerular capillary wall consists of fenestrated endothelium, the glomerular basement membrane, and the interdigitated foot processes of podocytes expressing the transmembrane protein nephrin.[5] Under physiological conditions, the structural integrity of this filtration barrier prevents the abnormal passage of albumin (molecular mass 66 kDa) and high-molecular-weight proteins (>66 kDa), whereas low-molecular-weight proteins (<66 kDa) can pass without restriction. In addition, the transglomerular passage of macromolecules is regulated by the charge-selective properties of the

glomerular capillary membrane and the hemodynamic forces operating across the capillary wall.[6;7]

In a healthy subject the amount of albumin excreted with urine normally represents less than 1% of the albumin filtered at the glomerular level.[8] The remaining albumin is reabsorbed predominantly by the proximal tubuli through cellular mechanisms, by means of the synergistic receptors megalin and cubilin.[9] This mechanism usually works quite close to saturation. As a consequence, any further increase in the amount of albumin filtered at the glomerular level will inevitably be accompanied by an increase in UAE.

In subjects with renal disease proteinuria is a good marker for renal disease progression.[10;11] However, in renal disease urinary protein loss usually results from a specific lesion within the kidney, not per se reflecting the "health" status of the overall vasculature. Therefore, it remains unclear whether in these patients UAE can be utilized as a predictor of cardiovascular risk.
In hypertensive and diabetic patients albumin leakage is more often considered to be a reflection of generalized endothelial or vascular dysfunction,[12;13] which is supported by the observation that elevated UAE is associated with elevated levels of high sensitivity C-reactive protein, von Willebrand factor and with impaired arterial dilatory capacity.[14-16] It is hypothesized that damaged endothelium may lead to increased leakage of plasma albumin and lipids through the vessel walls and evoke an inflammatory response, thus linking elevated UAE to atherosclerosis.[17] Since this process is not restricted to the kidney, microalbuminuria may be an indication of widespread vascular leakage of albumin. In addition to this hypothesis, increased intraglomerular pressure can also cause local renal leakage of albumin.

The evidence that albumin leakage is associated with cardiovascular risk is overwhelming. In recent studies on the potential prognostic value of microalbuminuria for cardiovascular events, the threshold value indicating increased risk has even been found to be well below the UAE values presently defining microalbuminuria regardless of the population included.[18] A continuous cardiovascular risk spectrum has been observed starting from albumin excretion rates as low as 10 mg/24h.[19;20]

Subsequent clinical evidence documented an association between UAE and other cardiovascular risk factors, target organ damage and risk of cardiovascular disease in the general population,[20-23] and in specific clinical contexts, including essential hypertension.[19;24;25] Accordingly, recent evidence suggests that reduction of UAE is associated with a lower cardiovascular risk.[26;27]

Urinary albumin excretion and cardiovascular risk factors

Although UAE is a powerful predictor of cardiovascular morbidity and mortality, even today it continues to be unclear whether UAE is a risk factor per se, or merely a reflection of the effects of other risk factors (Figure 1). The fact is, that UAE is associated with biological factors like age and gender, but also often coincides with other risk factors, such as hypertension, insulin resistance, and increased levels of low-density lipoprotein.[28-30]

Hypertension

Over 30 years ago Parving et al. were the first to find an association between UAE and blood pressure in a hypertensive population.[31] Since then several authors have confirmed this finding, and even in patients with high-normal blood pressure the prevalence of microalbuminuria has been shown to be increased.[32] The prevalence of elevated UAE in hypertensive populations has been reported to be up to 46%, depending on the technique of measurement and the definition used.[33] Recent work suggests that hypertensive patients with increased UEA are more inclined to show early signs of other end-organ disease as well, including left ventricular hypertrophy and increased carotid artery thickness.[34] In addition, blood pressure lowering reduces UAE.

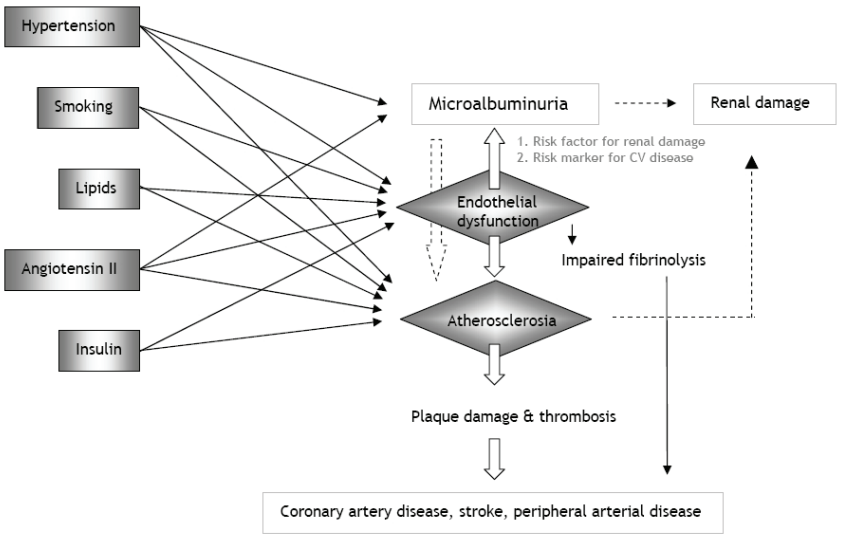

Figure 1. Possible factors involved in the pathogenesis of microalbuminuria (adapted from Verdecchia and Reboldi).[33]

The relation between insulin resistance and primary hypertension was described almost two decades ago.[46] Since then, several authors have investigated and discussed the association between insulin resistance and elevated UAE, but conflicting results were obtained.[47;48] In addition, it remains uncertain whether their presumed association is independent of other factors, such as obesity and hypertension.

Lipids

In patients with hypertension and diabetes, the combined presence of elevated UAE and hyperlipidemia is frequent. In diabetic populations several authors reported an association between UAE and atherogenic lipids.[49-51] In two non-diabetic hypertensive populations microalbuminuric subjects had a significantly worse lipid profile than their non-albuminuric counterparts.[52;53] In a large general population study, plasma cholesterol was an independent correlate of microalbuminuria.[54]

Several explanations for the association between hyperlipidemia and elevated UAE have been proposed.[53] First, the rise in serum lipids could be caused, direct or indirect, by the loss of proteins that are involved in lipid production. Second, lipid abnormalities may contribute to glomerulosclerosis by a mechanism similar to atherosclerosis.[55] In support of this theory is the finding that lipid-lowering medication also ameliorates UAE,[56] although these results are not supported by others.[26]

Smoking

Intrarenal hemodynamics can be influenced by smoking.[57] In smokers, the response of the kidney to increased systemic blood pressure may be impaired, possibly leading to increased intraglomerular capillary pressure.[58] The fact is, that smoking type I and type II diabetics excrete more albumin than non-smoking patients.[59;60] In non-diabetics, hypertensive or not, smoking also seems to be independently associated with elevated UAE, even in the range defined as normal.[61-63] For this reason, other non-hemodynamic mechanisms must be involved in the pathophysiology of smoking-induced albumin leakage. Importantly, damage induced by smoking is probably not limited to renal endothelium, but throughout the body the endothelial function may be affected. Although several mechanisms have been proposed (e.g. alteration of prostaglandin/thromoxane pathway, generation of reactive oxygen species, carbon monoxide-induced hypoxia, tubulotoxicity, increased clotting of platelets, increased creatinin resistance), the exact underlying mechanism remains to be determined.[64]

The underlying renal mechanism of hypertensive nephropathy has been the focus of research over the last years. At present, the common view is that due to failure of autoregulatory preglomerular constrictive response, the systemic hemodynamic load is conducted to the renal glomeruli.[35] The sympathetic nervous system may play a crucial role in this process.[36] Increased intraglomerular perfusion pressure will induce hyperfiltration and subsequently lead to a strain-related elevation of UAE.[37] Several studies have shown that agents that lower systolic and diastolic blood pressure reduce albuminuria, which establishes a strong argument in favor of this theory.[38-41] However, the finding that elevated UAE in hypertensive patients is an independent risk factor for cardiovascular disease, also suggests a link between vascular albumin leakage in the glomeruli, and systemic vascular damage. On the other hand, the pathogenesis of hypertensive nephropathy is multimodal and intrarenal mechanisms additional to blood pressure are involved, as the correlation with blood pressure can only partly explain urinary albumin loss. Interestingly, Brantsma et al recently reported that UAE may not always be merely the consequence of high blood pressure, but may also predict the development of hypertension.[42]

Diabetes mellitus and insulin resistance

UAE, as a component of diabetic nephropathy, is a common complication in diabetic subjects with a prevalence between 10 to 42%. In the early stages the patient will show hyperfiltration, represented by a high glomerular filtration rate, and the occasional occurrence of microalbuminuria. Later, a gradual decline can be observed, while microalbuminuria progresses to macro-albuminuria.[43] Accordingly, an association between elevated UAE and disease duration has been reported.

Hyperglycemia induces renal cells to produce cytokines and growth factors that are thought to be responsible for structural changes and for functional changes, such as increased permeability of the glomerular basement membrane. Several humeral factors have been proposed to contribute to these modifications, such as transforming growth factor-$ß_1$ (TGF- $ß_1$), platelet-derived growth factor, connective tissue growth factor, protein kinase C, and vascular permeability factor.[44] Advanced glycosylation end products, which are abundantly present in the diabetic kidney, have also been recognized to induce extracellular matrix production and hence contribute to glomerular sclerosis.[45] According to the Steno hypothesis, this detrimental process is not restricted to renal tissue, but will, to a certain degree, also affect extrarenal matrix components.[12]

The renin angiotensin system

The renin angiotensin system (RAS) is an endocrine pathway which provides a homeostatic control mechanism for sodium balance, intravascular volume, and therefore blood pressure (Figure 2).[65;66] The potent effector peptide, angiotensin II, is an octapeptide that is generated by cleavage from angiotensinogen through an action of two different peptides, renin and angiotensin converting enzyme (ACE).[67] The latter enzyme also links the RAS and the kallikrein-kinin system (KKS), as it breaks down bradykinin.[68]

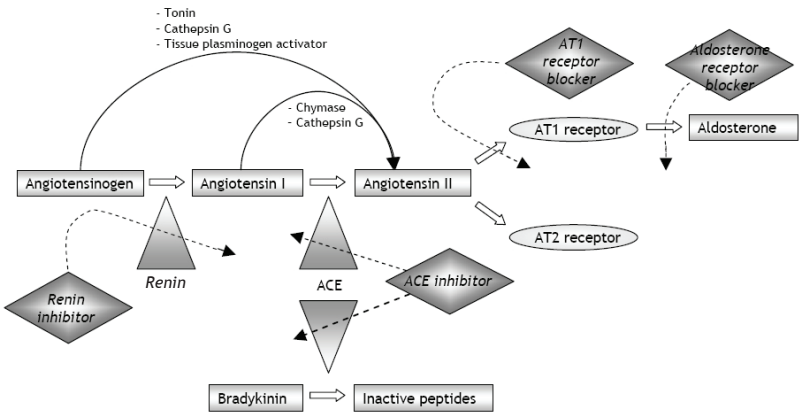

Figure 2. Simplified renin angiotensin system and targets for pharmacological intervention.

The role of angiotensin II in cardiovascular disease is well documented and nowadays this peptide is considered to be a key mediator of cardiovascular damage. The classical actions of angiotensin II include vasoconstriction, facilitation of sympathetic neurotransmission, and water and sodium retention. The latter can be accomplished directly or indirectly via aldosterone, a mineralocorticoid, which is considered to be an important mediator of angiotensin II-induced damage.[69]

Angiotensin II mediates its effects through two main receptor subtypes: the angiotensin II type 1 receptor (AT1R) and the angiotensin II type 2 receptor (AT2R). These two receptors are expressed and distributed heterogeneously throughout the human body, and can be found in peripheral tissues, several organs (e.g. in the kidney) and the brain.[70] Both receptors are seven transmembrane

glycoproteins with only 32-34 % homology and they both have been cloned.[71;72] The classical, in general detrimental, pheripheral actions of angiotensin II are mediated by the AT1R (Table 2). Although knowledge is expanding rapidly, the (patho)physiological role of the AT2R is still poorly understood. Due to these effects angiotensin II contributes to the pathogenesis of vascular, cardiac, renal and cerebral pathologies, such as atherosclerosis, post-infarction remodeling, left ventricular hypertrophy, heart failure, stroke, and possibly diabetes.

AT1 receptor	AT2 receptor
Vasoconstriction	Vasodilatation (?)
Na+ reabsorption/ H2O retention	Apoptosis
Renin suppression	Tissue regeneration/repair
Inotropic effects	Inhibition of tissue proliferation
Vascular and cardiac hypertrophy (TGF-ß$_1$)	Neuroprotection
Vascular injury and myocardial fibrosis	Cell differentiation
Proarrhythmic effects	Stimulation of bradykinin production
Prothrombotic effects (PAI-I ↑)	
Free radical formation (aging?)	
Facilitation of sympathetic transmission	
Endothelin secretion	
Proinflammatory effects	
Inhibition of Cell differentiation	
Facilitation of LDL transport	

Table 2. Angiotensin II mediated effects.
Abbreviations: TGF-β_1, transformin growth factor β_1; PAI-1, plasminogen activator inhibitor 1

The traditional view of the RAS being a hormonal system, whereby angiotensin II is exclusively formed in the circulation and transferred to peripheral tissues, has long been rejected.[73;74] A vast amount of evidence confirms that peripheral tissues are an important site of generation of this peptide, and local independent renin angiotensin systems have been found in many tissues, including brain, kidney, adrenal gland, pancreas, testis, blood vessels, and the heart.[70] Importantly, the autocrine and paracrine effects contribute to the regulation of local cellular and tissue functions, independent of the circulating endocrine system.[75] Furthermore, It is important to note that even within organs tissue RAS can be locally compartmentalized. For instance, within the kidney overall angiotensin II concentrations are higher in the medulla compared with the cortex.[76]

Renal and vascular endothelial effects of the RAS

RAS and Kidney

Sodium retention and excretion, resulting from aldosterone which is released after activation of AT1R in the kidney, provokes change of blood pressure and controls volume status. Other effects mediated via AT1R include regulation of endocrine functions, and stimulation of mitogenic pathways.

Angiotensin II exerts specific actions on intrarenal hemodynamics, thus contributing to a higher perfusion pressure. Angiotensin II-induced constriction of the efferent arteriole reduces renal blood flow and raises glomerular capillary pressure, consequently augmenting glomerular filtration rate and filtration fraction.[77;78] The changes in oncotic (↑) and hydrostatic (↓) pressure which are thus created in the peritubular vessels, are transduced to the interstitium, and promote a shift of sodium and water from the proximal tubule into the interstitium and systemic circulation. The reduction of medullary perfusion and diminished interstitial pressure simultaneously lower sodium and water excretion.[78] Beside these hemodynamic effects, the RAS increases water and sodium resorption by acting directly on several ion pumps (e.g. Na^+/H^+-antiporter, Na^+/K^+-ATPase, Na^+/HCO_3^--cotransporter) located throughout the tubular system.[79;80] Thus, the RAS plays a pivotal role in maintaining normovolumic state and a normal ion balance. However, derangement of this hormonal cascade is thought to induce renal dysfunction (Figure 3).

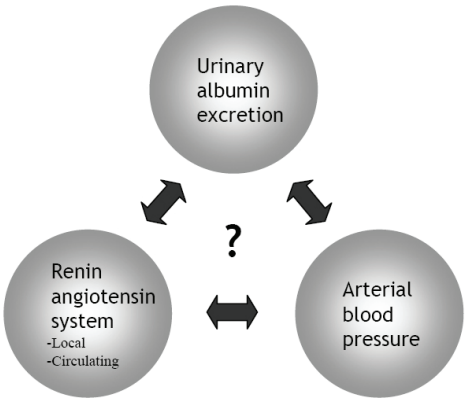

Figure 3. Our knowledge of the relation between the local and circulating renin angiotensin system, arterial blood pressure and urinary albumin excretion remains incomplete.

Furthermore, angiotensin II is responsible for the production of nephrotoxic oxygen species, profibrotic cytokins, and growth factors, consequently inducing cell proliferation and tissue remodeling. Together these effects promote the development of glomerulosclerosis and tubulointerstitial fibrosis.[81;82] Renal remodeling is augmented by angiotensin-induced aldosterone synthesis, as this hormone, besides being an important regulator of water and salt homeostasis, shares the ability to provoke mitogenic and profibrotic changes.[83] TGF-$ß_1$, plasminogen activator inhibitor-1, reactive oxygen species, endothelial dysfunction, upregulation of the AT1R have all been proposed as possible mediators of angiotensin II- and aldosterone-mediated renal injury and scarring (Table 2).[83] However, only few reports have been published showing a direct relationship between angiotensin II and glomerular permselectivity.[84;85]

The above-mentioned effects may induce and maintain glomerular leakiness, and therefore play a role in UAE. Conversely, urinary albumin may also stimulate the RAS in proximal tubular cells.[86]

Hemodynamic effect	Non-hemodynamic effect
Increased glomerular capillary pressure:	Induction of renal hypertrophy and cell proliferation
1. Post glomerular vasoconstriction (direct or indirect via endothelins ↑or NO ↓)	Modulation of extracellular matrix synthesis (↑) and degradadation (↓)
2. Systemic hypertension	Stimulation of cytokine (e.g. TGF-$ß_1$, VEGF, PAI-1)
Filtration surface area reduction due to mesangial cell contraction	Production of free oxygen radicals

Table 3. Summary of proposed angiotensin II-mediated mechanisms leading to increased urinary albumin excretion (adapted from Leehey et al.[87]).
Abbreviations: TGF- $ß_1$, transforming growth factor-$ß_1$; VEGF, vascular endothelial growth factor; PAI-1, plasminogen activator inhibitor-1; NO; nitric oxide

RAS and vascular endothelium

Angiotensin II exerts its proinflammatory effects through the AT1R (Table 2).[88] One of the most important, angiotensin II-modulated steps, in the initial stage of inflammation is the increase in vascular permeability.[89] Increased permeability of the vascular endothelium probably results from pressure-mediated mechanical injury, but also from angiotensin II-induced second mediators that may influence permeability (VEGF, prostaglandins).[88] This important early manifestation of inflammation and atherosclerosis will subsequently lead to cell infiltration and

exudation of protein-rich fluid. In line with this theory, an association between plasma renin activity and UAE was described in a small study performed in a population with essential hypertension.[90] In addition, a few authors found an association between the angiotensin converting enzyme DD genotype and the presence of elevated UAE.[91-93] For other genetic variants within the RAS (AGT M235T, and AT1 A1266C gene polymorphisms) this relationship remains to be determined.[93;94]

Pharmacological inhibition of the RAS in renal dysfunction

The cumulative incidence of diabetic nephropathy after duration of either type I or type II diabetes of 25 years has been reported to be 25-40%.[95] As diabetic and hypertensive nephropathy are the leading causes of end-stage renal disease in Europe and the United States, it is important to prevent, or at least to delay, in such subjects disease progression. Moreover, in non-diabetics the degree of UAE is related to cardiovascular prognosis. Considering this and the rising incidence of type II diabetes mellitus and its complications, it is imperative to develop new and to optimize old pharmacological strategies. Subsequently, the level of UAE may be used to monitor treatment efficacy,[96] although heterogeneity of changes in UAE during treatment may complicate the interpretation of efficacy.

ACE inhibitors

The antihypertensive effects of the first compound blocking the RAS were described in the early 1970s.[97;98] A few years later the first orally available ACE inhibitor, captopril, was marketed. Nowadays, more than 10 different ACE inhibitors are available for human use, all having specific pharmacological properties. ACE inhibitors block circulating and tissue ACE, and therefore lower systemic and local angiotensin II production and raise bradykinin levels (Figure 1). Besides lowering blood pressure, ACE inhibitors have clearly demonstrated to reduce proteinuria in both diabetic and non-diabetic nephropathy.[99] Several meta-analyses, suggest that this antiproteinuric effect is partly independent of blood pressure reduction, and greater than can be achieved with calcium blocking agents, diuretics and beta-blockers.[99] This suggests additional RAS-related effect up-and-above hemodynamic effects.

The Diabetic Nephropathy Trialist Group published a meta-analysis in which they demonstrate that ACE inhibitors significantly slow down the progression from microalbuminuria to macroalbuminuria in (type I) diabetic patients.[100]

In patients with overt nephropathy of type 1 diabetes, ACE inhibitors not only lowered UAE, but in addition significantly delayed the decline in glomerular filtration rate that is commonly observed in diabetic patients suffering from nephropathy.[101;102]

Data from the diabetic population (primarily type II diabetes mellitus) of the Heart Outcomes Prevention Evaluation (HOPE) trial suggested the ACE inhibitor ramipril, compared with placebo, significantly reduced the risk of developing nephropathy in these subjects.[103] Recently, the Bergamo Nephrologic Diabetes Complications Trial (BENEDICT) indicated that in hypertensive, normo-albuminuric type II diabetics the ACE inhibitor trandolapril was even more effective in preventing the development of microalbuminuria than the non-dihydropyridine calcium channel blocker verapamil.[104] In contrast, a small study in hypertensive type II diabetics did not reveal a significant difference between the antialbuminuric effects of ramipril and lercanidipine.[105]

ACE inhibitors have a clear renoprotective effect in non-diabetic renal disease, when compared to compounds not interfering with the activity of the RAS.[106] In patients with non-diabetic renal disease use of ramipril was even associated with an improvement of glomerular filtration rate.[107] The renoprotective effects of ACE inhibitors are probably mediated by their specific ability to block angiotenin II-induced constriction of the post-glomerular capillaries. The resulting fall in glomerular capillary pressure consequently reduces glomerular filtration rate and filtration fraction.

Beside their specific renoprotective effect, ACE inhibitors also protect the cardiovascular system. There is much debate whether ACE inhibitors offer more cardiovascular protection than the older blood pressure lowering agents, diuretics and beta-blockers. The meta-regression analysis by Staessen et al suggests that the efficacy of blood pressure lowering agents to prevent cardiovascular accidents and myocardial infarction is predominantly dependent on the reduction in blood pressure that is achieved.[108] Some recent individual studies suggests however, that ACE inhibitors may confer a cardiovascular protective effect that goes beyond blood pressure reduction per se.[103;109-112] Interestingly, a number of post-hoc analyses attributed the superior CV effect of ACE inhibition to be dependent on the level of albuminuria, for instance in the HOPE study, in which patients at high risk for cardiovascular events were randomised to either ramipril or placebo. Subgroup analysis of this study showed that in those patients with high baseline UAE, intervention with an ACE inhibitor is of particular value and improves cardiovascular prognosis.[103] The potency of

ACE inhibitors to induce cardiovascular benefits is also illustrated by the results from the Prevention of Vascular and Endstage Renal Disease Intervention Trial (PREVEND-IT). In this trial performed in non-hypertensive microalbuminuric subjects, fosinopril reduced UAE significantly and in addition, treatment was associated with a trend in reducing cardiovascular events.[26] Interestingly, the efficacy of fosinopril in this study to prevent cardiovascular events was shown to be dependent on baseline UAE. In subjects with UAE>50 mg/24hr the ACEi induced a 60% relative risk reduction in CV endpoints, whereas in subjects with baseline UAE 15-50 mg/24hr almost no cardioprotective effect was noted.

By blocking angiotensin II generation, ACE inhibitors induce a postglomerular vasodilation as stated above. This will reduce intraglomerular pressure and may consequently lead to a reversible reduction of GFR. In turn, this will lead to renoprotection in the long run ("short term pain, is long term gain"). Unfortunately, this may force a small selection of patients to suspend or discontinue therapy. Hypotension, cough and hyperkaliemia are relatively common side effects, whereas angioneurotic edema is a rare but potentially dangerous side effect of ACE inhibitors.
In summary, ACE inhibitors have demonstrated to be valuable antiproteinuric and renoprotective agents in both diabetic and non-diabetic subjects. Over the last few years evidence for their cardiovascular protective effects is mounting as well. Some evidence even suggests that these agents exert an extra beneficial cardiovascular profile in comparison to other blood pressure lowering agents, especially in albuminuric subjects.

Angiotensin II receptor blockers

The first peptide angiotensin type I receptor blockers (ARB), salarazin, was described in 1971,[113] but it took another 20 years before the first non-peptide ARB, losartan, became available for patient use. Since then, several ARBs have proven to lower blood pressure, slow down the progression of diabetic and non-diabetic renal disease, reduce proteinuria irrespective of the type of renal disease, and also reduce the risk of overt nephropathy.[114;115]

	Treatment	Patients	Follow up	Primary endpoint	Outcome
Parving HH, 2001	Irbesartan 150 mg 300 mg Placebo	590 patients, MA Hypertension	2y	Time to onset diabetic nephropathy	I150 vs P HR=0.56 I300 vs P HR=0.32
Lewis EJ, 2001	Irbesartan 200 mg Amlodipine 10 mg Placebo	1715 patients Proteinuria Hypertension	2.6y	Doubling serum creat Development of ESRD Death from any cause	I vs A RR=0.77 I vs P RR=0.80
Brenner MB, 2001	Losartan 50-100 mg Placebo	1513 patients Proteinuria	3.4y	Doubling serum creat Develoment of ESRD Death from any cause	L vs P RR=0.84
Viberti G, 2002	Valsartan 80 mg Amlodipine 5 mg	332 Patients, MA	1y	% Change UAE from baseline	V:56% UAE ↓ A: 8% UAE ↓

Table 4. Randomized controlled trials performed with angiotensin II receptor blockers in type II diabetic patients.
Abbreviations: MA; microalbuminuria, creat; creatinine, ESRD; end-stage renal disease, HR; hazard ratio, RR; relative risk

In contrast to ACE inhibitors, the evidence for a beneficial ARB-induced renoprotective effect in type 1 diabetic patients is limited.[116] Although ACE inhibitors and ARBs seem to have comparable effects on renal hemodynamics,[117] only small randomised placebo-controlled trials have actually studied the renoprotective properties of ARBs in type 1 diabetes.[118]

In type 2 diabetic patients, however, the evidence is compelling. Four large randomised clinical trials (IRMA-2,[119] IDNT,[120] RENAAL,[121] MARVAL[122]) established the beneficial (dose-dependent) effects of ARBs on a variety of renal endpoints in early and more advanced diabetic nephropathy (Table 4). Data from several other studies support these results.[115] Importantly, treatment with an ARB is not inferior to ACE inhibition, when renoprotective efficacies are compared.[123]

The antiproteinuric effects of ARBs in early non-diabetic hypertensive nephropathy have been described in animal experiments.[124;125] Initially, only smaller randomised clinical trials were performed in hypertensive non-diabetic subjects with[126] and without advanced renal disease.[127-132] Later, post-hoc analysis of a larger cohort of 918 patients with isolated systolic hypertension demonstrated that telmisartan reduced UAE irrespective of baseline UAE,

and to a greater extend than hydrochlorothiazide.[133] The most convincing evidence was provided by the Losartan Intervention For Endpoint Reduction in Hypertension (LIFE) study.[134] In 8602 hypertensive patients with ECG-ascertained left ventricular hypertrophy, treatment with losartan significantly reduced UAE. In addition, the reduction was significantly greater than the one achieved with atenolol, as was the reduction in cardiovascular endpoints. The authors concluded that part of the mechanism behind the superiority of losartan is related to its greater UAE-reducing effect.[134]

Interestingly, ARBs seem to have pleiotropic effects. For example, telmisartan has the ability to interact with the nuclear peroxisome proliferator-activated receptor gamma (PPAR-γ),[135] and olmesartan has been reported to exhibit anti-inflammatory effects in hypertensive patients.[136] The clinical value of these findings needs further exploration.

In summary, both ACE inhibitors and ARBs are first choice components of an antihypertensive treatment regimen in patients with diabetic or non-diabetic renal disease with albuminuria.[114;137] However, the predicted specific cardiovascular benefits of RAS-inhibitors in terms of survival and morbidity in subjects with renal damage need further verification.

Aldosterone receptor antagonists

The role of aldosterone in the development and progression of renal injury has been studied in variety of experimental models of progressive (hypertensive) renal disease.[138-140] Rodent experiments suggest that local overproduction of the mineralocorticoid receptor may be a significant factor in the predisposition to hypertension and the subsequent vascular and renal injuries.[141] In agreement with these findings, patients with an aldosterone-secreting tumour often have proteinuria.[142] Furthermore, several studies provide evidence that spironolactone, a non-selective aldosterone receptor antagonist, has a renovascular protective effect.[143-145] Spironolactone also improved left ventricular remodeling in patients experiencing a first anterior myocardial infarction,[146] and reduced cardiovascular mortality in severe chronic heart failure patients.[147] Recent data suggest that spironolactone may also improve acetylcholine induced vasodilation and thus protect endothelial function in patients with mild (NYHA class I or II) heart failure.[148] However, due to the lack of selectivity for the mineralocorticoid receptor and its endocrine side effects, the widespread use of spironolactone in humans is limited.

The selective aldosterone receptor antagonist eplerenone has recently demonstrated to have renoprotective properties that go beyond its antihypertensive effects as well. Compared with enalapril[149] and amlodipine,[150] the antiproteinuric properties of eplerenone were significantly better. In addition, combination of eplerenone and enalapril reduced UAE even to a greater extent than therapy with either drug alone.[149] However, whether this translates into long-term cardiovascular protection remains to be established. Although eplerenone has less antiandrogenic side effects than spironolactone, both drugs increase serum potassium, especially in patients using an ACE inhibitor or an ARB. Ensuing hyperkaliemia, although troublesome, is reversible, and depends on dose and creatinine clearance.[151] The fact that eplerenone is better tolerated than spironolactone offers the possibility to use eplerenone alone, or combined with an ACE inhibitor or ARB as a new renoprotective strategy. In the EPHESUS trial eplerenone demonstrated to improve post-infarction left ventricular remodeling, and subsequently reduce cardiovascular mortality.[152] Initial reports, although performed in rodents, suggest that the cardioprotective properties of this agent may be mediated, in part, by stimulating endothelial NO synthase.[153] Extrapolation of these results to patients without heart failure is complex, and currently unfeasible. However, these data again indicate that agents that interfere in the renin-angiotensin-system may result in reno- and cardiovascular protection that goes beyond what might be expected from blood pressure lowering alone.

Renin inhibitors

The latest addition to the RAS-blocking armamentarium are the renin inhibitors.[154] After a few disappointing predecessors, Aliskiren (SPP100) seems to be the first potent orally active alkane carboxamide renin inhibitor and the first reports suggest that its blood pressure lowering effects are comparable to other RAS blocking agents.[155;156] In addition, aliskiren is well-tolerated.[155] By actively antagonizing the rate-limiting step of the RAS cascade a general down-regulation of RAS activity can be observed.[156-158] Interestingly, Pilz et al. demonstrated that both low and high dose aliskiren reversed albuminuria and normalized serum creatinine in transgenic rats.[159] New renin inhibitors may therefore become an alternative to ACE inhibitors and ARBs in the treatment of hypertension, and possibly also provide end organ protection. Other renin inhibitors, for example zalkiren,[160] are currently under investigation. To date, none of these agents has proven to be cardioprotective.

Conclusion

Increased UAE is a common finding in diabetic patients, as well as in hypertensive patients without renal disease, which negatively affects prognosis. Even in the general population subjects with increased UAE experience more cardiovascular events than subjects with normal UAE. Although increased UAE seems to be interrelated with several other cardiovascular risk factors and markers, evidence emerges that albuminuria is also an independent risk factor. As blocking the RAS induces a specific reduction of UAE that is greater than might be expected from blood pressure lowering alone, a causal relationship between RAS and UAE is suggested. Beside ACE inhibitors and blockers of the AT1R, (selective) aldosterone receptor antagonists and renin inhibitors may also be able to reduce UAE. Yet, whether the efficacy of these compounds to lower albuminuria is a specific renal mechanism, or a reflection of a superior efficacy of these drugs to ameliorate generalized endothelial dysfunction is yet unknown. Recent evidence suggests however, that these compounds may have a specific cardioprotective effect in subjects with higher levels of albuminuria, besides their proven renoprotective value.

Outline of this thesis

This present thesis concerns dysfunction of the cardiorenal axis, with a special focus on elevated urinary albumin excretion and the renin angiotensin system. The effect of pharmacological blockade of the renin angiotensin system on the activity of the renin angiotensin system and on urinary albumin excretion will also be discussed.

The value of pre-operative renal function as a predictor of long-term outcome in patients undergoing coronary artery bypass graft surgery will be described in **Chapter 2.** In **Chapter 3** we describe the prevalence of microalbuminuria in a cohort of severe chronic heart failure patients. We also present the neurohormonal variation that coincides with elevated urinary albumin excretion in these patients. The renin angiotensin system can be blocked by ACE inhibitors and angiotensin II type 1 receptor blockers. **Chapter 4** elaborates on the renin angiotensin system in chronic heart failure patients and the effect of ACE inhibition on the activity of this cascade. One of the benefits attributed to angiotensin II type 1 receptor blockers is parallel stimulation of the angiotensin II type 2 receptor. For this reason, we analyse the vasoactive effects of the type 2 receptor in human arteries in **Chapter 5**. Several studies suggest that combination of angiotensin II type 1 receptor blockers and ACE inhibitors can provide a more complete protection against the detrimental effects of the renin angiotensin system. In **Chapter 6** we discuss the pros and cons of this strategy. Finally, in **Chapter 7** we present data from the PREVEND intervention trial. In otherwise healthy, microalbuminuric subjects we determine which parameters affect baseline urinary albumin excretion. In an additional analysis we verify the antialbuminuric effect of ACE inhibition, and we describe which parameters determine the efficacy of treatment.

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